dramatically upon the introduction of mutations that bias EmrE orientation. While early mutations, especially those surrounding the first transmembrane helix, appear capable of biasing the initial orientation, the effects of later mutations can be explained by altering the rate of misfolding events in accordance with the positive-inside rule. Positive charges thus appear to act as folding signals, enhancing proper folding and insertion when positioned in agreement with the positive-inside rule, and enhancing misfolding when placed in violation.

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Developing a Universal Steric Trapping Strategy for Studying Folding and Stability of Helical Membrane Proteins

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"Steric trapping" is a method that links binding of monovalent streptavidin (mSA) to unfolding of a biotinylated protein (MP). It allowed the measurements of high affinity protein–protein interactions and thermodynamic stability of polytopic helical MPs in a native environment, which had been difficult to achieve using more conventional methods. In the current steric trapping framework, a conformation-sensitive chromophore or an enzymatic activity in a target MP is required for monitoring mSA-induced unfolding. This target-specific approach is a limiting factor that hinders its application to various MP systems, *i.e.* MPs in a misfolded conformation, MPs with an assembly role, or MPs without convenient unfolding readout. To further advance the steric trapping strategy for more general application, we have developed novel tripartite probes possessing a thiol-reactive group, a biotin group and a fluorescent or paramagnetic group for sensitization of unfolding or binding of mSA. We applied the new strategy to investigating the stability and unfolding mechanism of an intramembrane protease GlpG. By combining FRET between fluorescently labeled GlpG and quencher-labeled mSA as a measure of mSA binding and the proteolytic activity of GlpG as a measure of unfolding, we proved the thermodynamic coupling between binding and unfolding, and determined the thermodynamic stability and unfolding rate of GlpG in a native micellar environment. While the stabilities were similar independent of the location of biotin pairs, the unfolding was 20 times faster when the biotin pair was placed near the proteolytic active site. This result suggests a subdomain-organization of the helical bundle architecture of GlpG. Steric trapping may serve as a useful tool for elucidating the local versus global flexibility of helical MPs.

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Studying Membrane Protein Folding by Molecular Dynamics Simulations

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Membrane protein folding is a process of fundamental importance in biophysics and structural biology. Despite major advances in our understanding of translocon-mediated insertion of membrane proteins, many aspects of the underlying biophysics remain unclear.

Computational methods provide a powerful tool for understanding membrane protein folding. As an initial model for a translocon tunnel, we are using a simplified pore system to investigate systematically the effects of this on helix formation. This approach is being used in parallel with enhanced sampling methods to accelerate the folding reaction. As test systems we have focused on two membrane proteins: the influenza M2 channel protein which contains a single transmembrane (TM helix plus a C-terminal amphipathic helix, and the seven TM helix protein bacteriorhodopsin, For bacteriorhodopsin we can successfully fold all seven helices inside our simple model of a translocon, starting from a fully extended conformation. For influenza M2, we observe the two helices (TM and amphipathic) folding to a state that is consistent with NMR structures.

We wish to develop this model further to include the crucial step of helix insertion into the membrane. In this context we are also exploring coarse-grained approaches to help us address time-scales accessible to simulations.

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Identifying the Oligomerization State of DegP in the Absence and Presence of Substrate

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Bacterial outer membrane proteins (OMPs) are synthesized in the cytoplasm, cross the inner membrane, and are then transported across the periplasm before folding into the outer membrane. Similar pathways are present in both mitochondria and chloroplasts. While unfolded outer membrane proteins (uOMPs) are prone to aggregation, they are able to avoid this off pathway reaction with the help of periplasmic chaperones and proteases. The primary protease that interacts with uOMPs is DegP. DegP is a member of the high temperature requirement A (HTRA) protease family and has been implicated in both the Cpx and σ^E stress response pathways. DegP sequesters and degrades uOMPS when they accumulate in the periplasm. It has been suggested that DegP functions by transitioning from an inactive hexameric state to an active cage-like oligomeric state of either 12 or 24 subunits when substrate is present. In order to further investigate the relationship between the oligomeric state of DegP and the presence of substrate, we performed sedimentation velocity experiments with and without various uOMPs. This allows us to identify the oligomeric populations of DegP at biological concentrations. Results suggest that DegP has significant populations of multiple oligomeric states even in the absence of substrate. This work contributes to our understanding of OMP biogenesis by identifying the nature of uOMP interactions with an important pathway component.

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Theoretical Prediction of Mutations Improving Thermal Stability of Adenosine A2a Receptor

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G protein-coupled receptors (GPCRs) are physiologically important membrane proteins possessing seven transmembrane domains, which are responsible for signal transduction pathways. Therefore, they form the most important target for drug design. However, their mass production and structure determination by the X-ray crystallography are quite difficult to achieve due to the low thermal stability in detergents. Though the stability can be enhanced by introducing mutations into GPCRs, a random search accompanying a heavy experimental burden is currently employed to obtain mutations leading to sufficient enhancement. In the present study, through mutations for the antagonist-binding structure of the adenosine A2a receptor, we investigate how to predict the mutants which lead to enhanced thermal stability using our free-energy function (FEF) recently developed for membrane proteins. The FEF comprises two components: the energetic term, which is focused on the energy decrease arising from formation of intramolecular hydrogen bonds, and the entropic term, which originates from the translational displacement of hydrocarbon groups constituting nonpolar chains of the lipid bilayer. After calculations of the FEF for all mutants, we have chosen some candidate mutants whose thermal stability would be most improved, and then their stabilities are experimentally examined. The findings are as follows. The success rate of the prediction focused on the entropic term alone is about 1/3 that is much higher than that reached by the trial-and-error prediction. This result implies that the entropic effect of hydrocarbon groups is critical for the structural stability of GPCRs. Moreover, when the energetic term is also considered, the success rate is improved to 1/2. Since the calculation of the FEF can be accomplished quite rapidly, we can theoretically examine a large number of mutations.

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Bioinformatic Methods for the Rapid Identification of Thermostabilizing Mutants

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Membrane proteins are often of particular difficulty to study, frequently limited by stability in detergent solution. Taking advantage of the available genomic and cell culture data, presented are two bioinformatic methods to quickly identify a limited set of amino acids or positions which likely underlie the thermal adaptation of a given protein family. This set then provides a small number of mutants to screen for stabilization and are used to demonstrate a significant increase in thermostability of an example membrane protein.

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Interaction of the Phage Endolysin PlyC with Model Membranes

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Endolysins are bacteriophage-encoded peptidoglycan hydrolases expressed during the late stages of a phage replication cycle that function to lyse the